Novel Systems for the Production of Rare Sugars and Oligosaccharides

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Novel Systems for the Production of Rare Sugars and Oligosaccharides

Who is zuChem?
Industrial Biotechnology Company:
- Platform of Process Technology for Food and Pharma

Product Focus:
- Value-Added Monosaccharides:
  - Polyols – Mannitol/Xylitol natural fermentative process
  - Xylitol currently in pilot
  - Pharma intermediates – Rare Sugars

Activated Sugar Precursors
- Glycoconjugates
- Prebiotics/Oligosaccharide Prebiotics
- Pharma focused glycoconjugates

The Importance of Carbohydrates
Sugars key in Health and Nutrition

Over Half the World’s Drug Leads Derived From Natural Products:
- >78% Antimicrobials, Many anticancer
- A large number of these are glycosylated

Sugars are On the Surface of our Cells
- Bacteria and Virus entry points

Sugars involved in tissue targeting
- Delivery of molecules to different parts of body and cell

Sugars involved in Metabolic Control
- Regulate metabolism

Rare and Activated Sugar Technology

Rare Sugar Building Blocks for L-Nucleoside Pharmaceuticals

L-Ribose Production Method

R-ribose
Making Rare Sugars: L-Ribose and Others

- Gene cloned and expressed in E. coli

Engineered Enzyme for Improved Performance

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>MDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Mannitol</td>
<td>D-Mannose</td>
<td>100%</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>L-Arabinose</td>
<td>33%</td>
</tr>
<tr>
<td>Erythritol</td>
<td>L-Erythrose</td>
<td>4%</td>
</tr>
<tr>
<td>Ribitol</td>
<td>L-Ribose</td>
<td>50%</td>
</tr>
<tr>
<td>Galactitol</td>
<td>L-Galactose</td>
<td>65%</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>L-Gulose</td>
<td>10%</td>
</tr>
</tbody>
</table>

- Used Directed Evolution to Create Improved Mutant
- Yield of L-Ribose now over 81% > 50 g/L
- Capable of making a variety of rare sugars including L-Ribose, L-Galactose, L-Gulose and others

Making Activated Sugars

- Key Component for Sugar Attachment
- Basic 2-step biological Strategy:

Cloned Novel Kinase and Engineered for Relaxed Substrate Specificity

- Reaction monitored via ATP consumption by HPLC: Expressed as % conversion (% ATP consumption)

Cloned Novel Nucleotidytransferase and Engineered for Relaxed Substrate Specificity

- Activity at 37°C, stable up to 1 week
- Shows activity with UTP, GTP and TTP
- Mutation introduced to increase enzyme activity toward galactose.

Production of UDP-Gal Activated Sugar in vitro

- Galactose-1-phosphate yield after purification 120 g/L
- UDP-Galactose yield 70 g/L
**Range of Activated Sugars and Intermediates**

<table>
<thead>
<tr>
<th>Sugar 1-Phosphates</th>
<th>Activated Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-galactose-1P</td>
<td>UDP-D-galactose</td>
</tr>
<tr>
<td>2-deoxy-D-galactose-1P</td>
<td>GDP-D-galactose</td>
</tr>
<tr>
<td>6-azido-6-deoxy-D-gal-1P</td>
<td>GDP/TDP/UDP-D-glucose</td>
</tr>
<tr>
<td>D-fucose-1P</td>
<td>GDP/TDP/UDP-L-glucose</td>
</tr>
<tr>
<td>L-glucose-1P</td>
<td>GDP/TDP/UDP-2-deoxy-D-galactose</td>
</tr>
<tr>
<td>L-xylose-1P</td>
<td>TDP-D-fucose</td>
</tr>
<tr>
<td>D-galactosamine-1P</td>
<td>TDP-D-fucofuranose</td>
</tr>
<tr>
<td>D-arabinose-1P</td>
<td>TDP-4-keto-2,6-di-deoxy-D-glucose</td>
</tr>
<tr>
<td>D-glucose-1P</td>
<td>TDP-D-olivose</td>
</tr>
<tr>
<td>D-xylose-1P</td>
<td></td>
</tr>
<tr>
<td>D-glucosamine-1P</td>
<td></td>
</tr>
<tr>
<td>D-mannose-1-P</td>
<td></td>
</tr>
</tbody>
</table>

**Activated Sugars**

- UDP-D-galactose
- GDP-D-galactose
- UDP-GlcNAc
- GDP/TDP/UDP-D-glucose
- GDP/TDP/UDP-L-glucose
- GDP/TDP/UDP-2-deoxy-D-galactose
- TDP-D-fucose
- TDP-D-fucofuranose
- TDP-4-keto-2,6-di-deoxy-D-glucose
- TDP-D-olivose

**Example: Oligosaccharides**

**Synthesis of Globotriose**

Galactosylation reaction catalyzed by the LgtC glycosyltransferase

**Production of Globotriose**

- Intermediates Required:
  - Lactose
  - UDP-D-galactose
- Reaction:
  - Completed in 4h with a yield of 62 g/L
  - Recent optimizations show yield of over 200 g/L

**First Large Scale Commercial Product: Xylitol**

- Hemicellulose inexpensive abundant supply mixed C5/C6 is OK
- Fermentation (proprietary)
  - Xylitol no polyol contaminants simple purification
- Advantages
  - Relieves Feedstock Constraints
  - Uses a wide variety of renewable feedstocks
- Commercial Partner
  - Godavari BioRefineries
  - Product Samples this Year, Commercial 2015
Conclusions

Novel Set of Enzymes Developed
- Produce rare sugars, sugar-1-phosphates, and activated sugars
- Stable enzymes with high expression in E. coli
- Engineered ability to work with broad range of substrates

Can now produce large variety of sugar-1-phosphates, activated sugars
- Broad range
- High yield
- Available as R&D Reagents

Demonstrated Scale-up of Oligosaccharides
- Can produce 100s grams of several oligosaccharides
- Now scaling PMAD technologies – Establishing Commercial partnerships
- Moving systems in vivo for larger scale production

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zuChem
Enabling Carbohydrate Chemistry!

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